insect, the tobacco hornworm *Manduca sexta*. Further support comes from Taniai *et al.* [16], who found that hemocytes from the silkworm, *Bombyx mori*, release lipopolysaccharides from phagocytosed bacteria, and that the presence of such bacterial components correlates with an increased immunostimulatory activity of a hemocyte supernatant.

The latter model predicts that the hemocytes have an efficient mechanism for the export of digestion products, such as peptidoglycan fragments, from phagocytosed microorganisms. That would be an interesting parallel to the antigen-presenting cells of the acquired immune system in vertebrates. Future work will reveal whether *Drosophila* hemocytes are 'antigen-presenting' cells, whether they act via cytokines, or in fact whether both mechanisms operate.

References

1. Bulet, P., Hetru, C., Dimarcq, J.L., and Hoffmann, D. (1999). Antimicrobial peptides in insects; structure and function. Develop. Comp. Immunol. *23*, 329–344.
2. Hultmark, D. (2003). *Drosophila* immunity: paths and patterns. Curr. Opin. Immunol. *15*, 12–19.
3. Hoffmann, J.A. (2003). The immune response of *Drosophila*. Nature *426*, 33–38.
4. Leclerc, V., and Reichhart, J.M. (2004). The immune response of *Drosophila melanogaster*. Immunol. Rev. *198*, 59–71.
5. Tanji, T., and Ip, Y.T. (2005). Regulators of the Toll and Imd pathways in the Drosophila innate immune response. Trends Immunol. *26*, 193–198.
6. Brennan, C.A., Delaney, J.R., Schneider, D.S., and Anderson, K.V. (2007). Psidin is required in *Drosophila* blood cells for phagocytic degradation and activation of the humoral immune response. Curr. Biol. *17*, 67–72.
7. Steiner, H. (2004). Peptidoglycan recognition proteins: on and off switches for innate immunity. Immunol. Rev. *198*, 83–96.
8. Kaneko, T., and Silverman, N. (2005). Bacterial recognition and signalling by the *Drosophila* IMD pathway. Cell. Microbiol. *7*, 461–469.
9. Royet, J., Reichhart, J.M., and Hoffmann, J.A. (2005). Sensing and signaling during infection in *Drosophila*. Curr. Opin. Immunol. *17*, 11–17.
10. Gateff, E. (1994). Tumor-suppressor genes, hematopoietic malignancies and other hematopoietic disorders of *Drosophila melanogaster*. In Primordial Immunity: Foundations for the Vertebrate Immune System, Ann. N.Y. Acad. Sci., *Volume 712*, G. Beck, E.L. Cooper, G.S. Habicht and J.J. Marchalonis, eds. (New York: New York Acad. Sci.), pp. 260–279.
11. Braun, A., Hoffmann, J.A., and Meister, M. (1998). Analysis of the *Drosophila* host defense in *domino* mutant larvae, which are devoid of hemocytes. Proc. Natl. Acad. Sci. USA *95*, 14337–14342.
12. Basset, A., Khush, R., Braun, A., Gardan, L., Boccard, F., Hoffmann, J., and Lemaitre, B. (2000). The phytopathogenic bacteria *Erwinia carotovora* infects *Drosophila* and activates an immune response. Proc. Natl. Acad. Sci. USA *97*, 3376–3381.
13. Foley, E., and O'Farrell, P.H. (2003). Nitric oxide contributes to induction of innate immune responses to gram-negative bacteria in *Drosophila*. Genes Dev. *17*, 115–125.
14. Uematsu, S., and Akira, S. (2006). Toll-like receptors and innate immunity. J. Mol. Med. *84*, 712–725.
15. Dunn, P.E., Dai, W., Kanost, M.R., and Geng, C. (1985). Soluble peptidoglycan fragments stimulate antibacterial protein synthesis by fat body from larvae of *Manduca sexta*. Develop. Comp. Immunol. *9*, 559–568.
16. Taniai, K., Wago, H., and Yamakawa, M. (1997). In vitro phagocytosis of *Escherichia coli* and release of lipopolysaccharide by adhering hemocytes of the silkworm, *Bombyx mori*. Biochem. Biophys. Res. Commun. *231*, 623–627.

Umeå Centre for Molecular Pathogenesis, By. 6L, Umeå University, S-901 87 Umeå, Sweden.
E-mail: dan.hultmark@ucmp.umu.se, karin.borge@ucmp.umu.se

DOI: 10.1016/j.cub.2006.11.039

Cell Adhesion: Separation of p120's Powers?

The catenin p120 is involved in many processes, including cell–cell adhesion and cancer. Recent work explores whether p120 independently regulates two key binding partners, RhoGTPase and cadherin.

Donald T. Fox[1] and Mark Peifer[1,2]

Regulation of cell–cell adhesion is critical for both morphogenesis and metastasis. Cell-adhesion complexes maintain and remodel tissues via linkage to the cytoskeleton. Regulated changes in adhesion are coordinated with cytoskeletal changes, thus directing cellular and tissue morphogenesis. Conversely, loss of cell–cell adhesion can promote tumor metastasis via changes in cell motility. Thus, examining regulators of adhesion and the cytoskeleton will advance our understanding of development and disease.

Despite its humble name, the p120 protein is extremely versatile, playing roles in adhesion, nuclear signaling, and cancer. p120 also binds to a formidable array of partners, including cadherins, which mediate cell–cell adhesion, and the cytoskeletal regulator Rho, a small GTPase. Understanding p120's multiple functions requires assigning particular functions to particular partners. While many p120 partners were initially identified *in vitro*, their *in vivo* relevance is now being addressed [1].

Several recent papers [2–5] argue that p120 regulates both cadherins and Rho in many cellular processes, but suggest that the question of whether p120 regulates these targets separately does not have a simple answer. p120 plays an important role at the interface of adhesion and cytoskeletal regulation during development and oncogenesis. Originally identified as a substrate of the Src oncogene, p120 was subsequently found to bind cadherins [1]. Loss-of-function studies confirmed that p120 promotes adhesion, at least in part, by inhibiting endocytosis of cadherins [6–9]. Recent *in vivo* work in mammals emphasized p120's importance in cadherin stabilization [2–5,10].

Overexpression studies identified another p120 target — RhoGTPase. p120 overexpression reduces cell contractility and actin-rich stress fibers, while increasing cell motility, at least in part, by inhibiting Rho and activating Rac and Cdc42 [11–13]. Rho can bind both p120 and α-catenin [14], suggesting that regulation might occur at cell junctions. However, E-cadherin

co-overexpression reverses p120's cytoskeletal effects, potentially by sequestering p120 at junctions. This suggests that p120 regulates adhesion and RhoGTPases independently [12,13]. Given the caveats with overexpression experiments, the physiological relevance of Rho regulation by p120 remained in question. Recent loss-of-function studies [2–5] significantly strengthen the idea that p120 regulates Rho, and examine whether this occurs independently of cadherin regulation.

p120 and Invasiveness

Coordinated regulation of adhesion and the cytoskeleton plays a key role in epithelial–mesenchymal transitions, both in embryogenesis and during metastasis, where adhesion is reduced while migration is stimulated. Recent work by Yanagisawa and Anastasiadis [3] in E-cadherin-deficient tumor cells revealed roles for p120 in motility and invasiveness. The absence of E-cadherin from these cells allowed for a clean separation between p120's roles in cytoskeletal and E-cadherin regulation.

p120 depletion using siRNA dramatically reduces tumor cell motility [3]. The absence of E-cadherin might suggest that this effect is adhesion-independent, but things are more complex. Normal migratory cells downregulate E-cadherin, but at the same time often upregulate mesenchymal cadherins. Similar changes have been shown to occur during metastasis [15,16]. Interestingly, Yanagisawa and Anastasiadis [3] found that knockdown of p120 destabilizes the mesenchymal cadherins N-cadherin and cadherin-11. Furthermore, knockdown of mesenchymal cadherins in E-cadherin-deficient cells suppresses invasiveness, thus mimicking p120 knockdown. This suggests that, in E-cadherin's absence, p120 stabilizes mesenchymal cadherins, which then promote invasiveness (Figure 1A). Thus, while p120 acts independently of E-cadherin, it still regulates the stability of other cadherins.

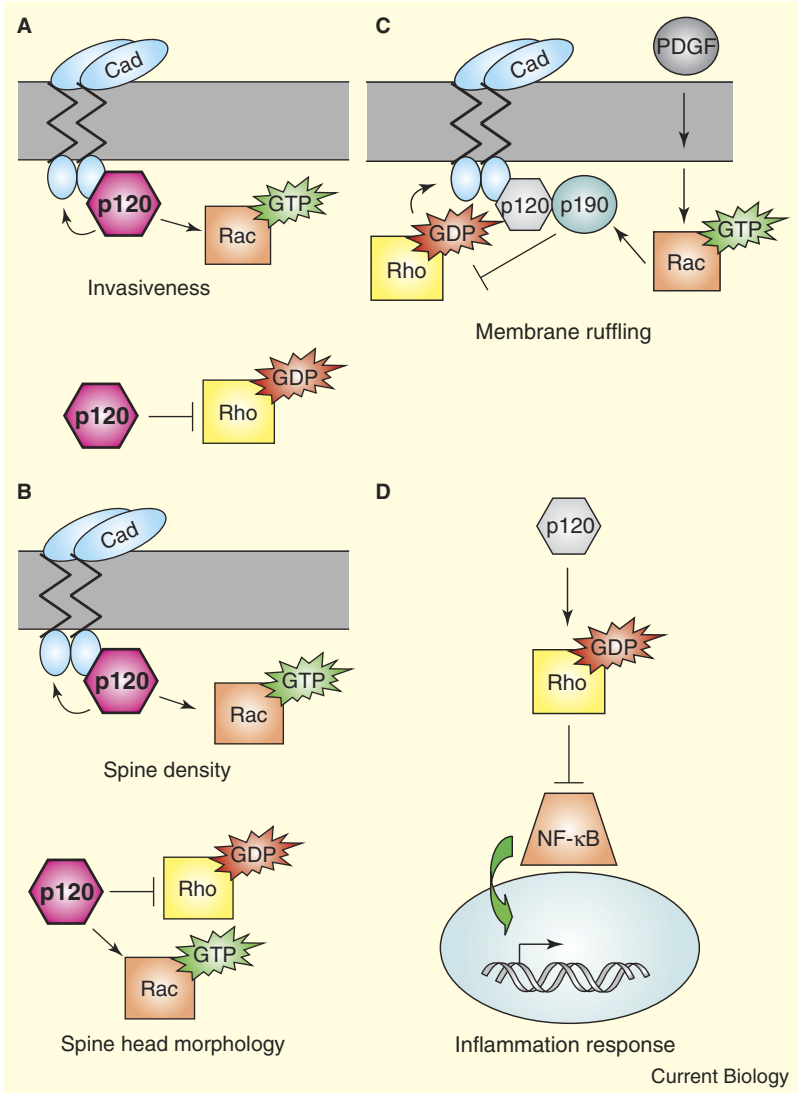

Figure 1. Models for p120 regulation of Rho and cadherins suggest diversity in functions.

(A) In E-cadherin-deficient cells [3], p120 promotes invasiveness through mesenchymal cadherin stabilization and Rac activation. A separate pool of p120 inhibits Rho. (B) In hippocampal dendrites [5], N-cadherin-bound p120 activates Rac while a separate pool of p120 activates Rac and inhibits Rho. (C) In fibroblasts [2], PDGF signaling activates Rac, which in turn activates the Rho inhibitor p190 RhoGAP (p190). p190's Rho inhibition is coupled to N-cadherin stability through p120 binding. (D) In the epidermis [4], p120 promotes an inflammatory response through Rho inhibition, preventing activation and translocation of NF-κB to the nucleus, which would trigger pro-inflammatory gene expression.

Yanagisawa and Anastasiadis [3] next examined two candidate p120 targets: Rac and Rho (Figure 1A). p120 loss decreases Rac activity and increases Rho activity [3], consistent with the known effects of p120 overexpression [12–14]. Rho and Rac function downstream of p120-mediated invasiveness, as experimental activation of Rac partially restores invasiveness in p120's absence, and invasiveness is further restored by Rho inhibition [3]. Yanagisawa and Anastasiadis [3] then asked whether p120 regulates these GTPases via mesenchymal cadherins. Administration of cadherin-11 siRNA to tumor cells blocks p120-dependent Rac activation, but not Rho inhibition. Thus, p120 regulates invasiveness in multiple ways by binding different targets; cadherin-bound p120 regulates

Rac, while a different p120 pool regulates Rho (Figure 1A).

p120 and Neuronal Morphogenesis

Neurons provide an *in vivo* example for the intimate interplay between adhesion and cytoskeletal regulation during morphogenesis. Elia *et al.* [5] conditionally knocked-out p120 in the mouse forebrain. Such mice are viable and do not show drastic brain defects, which is likely to be due to redundancy of several p120 paralogs. However, in the hippocampus, p120 loss dramatically affects the morphology of dendritic spines, actin-rich structures that collect synaptic input. Spine morphology and number have been shown to depend on cadherins and Rho GTPases [17]. In p120's absence, spine number and length are reduced, spine head morphology is altered, and dendrite branching decreases. These phenotypes are associated with changes in the levels and activity of familiar targets: without p120, Rac activity decreases and Rho activity increases. N-cadherin levels also decrease, albeit not dramatically, again perhaps due to p120 family redundancy.

Elia *et al.* [5] then explored which p120 targets are critical for dendrite formation (Figure 1B). A p120 mutant that cannot bind cadherin restores spine density but not head morphology, while a p120 mutant that cannot regulate Rho behaves oppositely, rescuing spine head morphology but not spine density. However, Rac activation by p120 also plays a role in both processes. These elegant experiments reveal roles for p120 that depend on cadherin but not Rho regulation (spine density), or on Rho but not cadherin regulation (head morphology).

p120 and Inflammation

A targeted p120 knockout in mouse skin revealed an unanticipated role for p120 during inflammation responses. When Perez-Moreno *et al.* [4] knocked-out p120 in the developing epidermis, they probably expected reduced E-cadherin levels and epithelial disruption, but, as in the forebrain, p120 loss doesn't disrupt morphology or barrier function of newborn skin. E- and P-cadherin levels decrease substantially, but the remaining cadherins, perhaps stabilized by p120 paralogs, are sufficient for normal skin morphology.

However, as these mice age, there is increased proliferation in the skin's basal layer, leading to epidermal hyperplasia. Surprisingly, this doesn't result from changes in epidermal integrity. Instead, it results from infiltration by immune cells, as pharmacological reduction of inflammation rescues the phenotype. Inflammation is triggered because p120 mutant keratinocytes activate NFκ-B and express cytokines (Figure 1D).

Perez-Moreno *et al.* [4] then examined which p120 target was involved (Figure 1D). Rho activity increases in p120 mutant keratinocytes, and active Rho activates NFκ-B in wild-type keratinocytes. Finally, a p120 mutant lacking the Rho-regulatory domain cannot repress NFκ-B, while p120 lacking the cadherin-binding domain can. Thus in the skin, p120 regulates both cadherin and Rho, and by inhibiting Rho prevents an NFκ-B-mediated immune response.

p120 and Growth Factor Signaling

While all of these papers suggest a relatively clean separation of p120-dependent regulation of Rho and cadherins, new work from the Reynolds lab [2] suggests this is not true in all situations. Wildenberg *et al.* [2] examined fibroblasts after treatment with platelet-derived-growth-factor (PDGF), which triggers dramatic cytoskeletal reorganization, creating membrane ruffles through a well-characterized pathway involving Rac and Rho. Surprisingly, application of p120 siRNA blocks this response. p120 inhibits Rho during ruffling, as p120 knockdown increases stress fibers and Rho activation, and inhibition of the Rho-target ROCK blocks p120 siRNA phenotypes. The researchers then looked upstream to determine at which point p120 fits into the PDGF pathway (Figure 1C). p120 is not upstream of Rac in these cells, but instead binds the Rho-inhibitor p190RhoGAP (p190), which is activated by Rac. Furthermore, PDGF stimulation recruits p190 and p120 to membrane ruffles and p190 recruitment to ruffles requires both p120 and Rac activation.

Wildenberg *et al.* [2] found connections between PDGF signaling and adhesion, as N-cadherin also co-localizes with p120 in ruffles (Figure 1C). p120 knockdown decreases N-cadherin stability. Finally, membrane-association of N-cadherin and p120 is reduced in p190 mutant cells. Thus, during PDGF-induced ruffling, p120's association with p190 both inhibits Rho and promotes cadherin-based adhesion. This suggests a model whereby p120 localizes Rho inhibition machinery to cadherins, which in turn stabilizes adhesion. Unlike the cases above, this suggests a strong connection between the regulation of Rho and cadherins by p120.

Taken together, these papers [2–5] strongly support physiologically relevant roles for p120 in the regulation of Rho activity. This may be an innovation of vertebrate p120, as the amino-terminal region critical for Rho regulation is lacking in *Drosophila* and in the worm *Caenorhabditis elegans* p120. *Drosophila* p120 is not an essential Rho regulator *in vivo* [6,7,18,19], but p120 mutants do show alterations in dendrite morphology [20]. p120's connections to cell–cell adhesion and the cytoskeleton suggest that this protein plays a key role in regulating decisions between epithelial and mesenchymal behavior. In one state, stable adhesion and active Rho inhibit motility, whereas in another state lowered adhesion and inactive Rho promote motility. Cadherin accumulation in response to PDGF-induced ruffling may represent an intermediate state, in which Rac activation and Rho inhibition increase the number of actin-based protrusions, which localize to nascent cell–cell contacts in many cell types. These data further suggest that

mammalian p120 regulates Rho and cadherins separately in some cases, while their regulation might be coupled in others. Of course other mammalian p120 paralogs add to the complexity. Future work will continue to unravel the complex relationship between p120, Rho, and cadherin.

References

1. Reynolds, A.B., and Roczniak-Ferguson, A. (2004). Emerging roles for p120-catenin in cell adhesion and cancer. Oncogene *23*, 7947–7956.
2. Wildenberg, G.A., Dohn, M.R., Carnahan, R.H., Davis, M.A., Lobdell, N.A., Settleman, J., and Reynolds, A.B. (2006). p120-catenin and p190RhoGAP regulate cell-cell adhesion by coordinating antagonism between Rac and Rho. Cell *127*, 1027–1039.
3. Yanagisawa, M., and Anastasiadis, P.Z. (2006). p120 catenin is essential for mesenchymal cadherin-mediated regulation of cell motility and invasiveness. J. Cell Biol. *174*, 1087–1096.
4. Perez-Moreno, M., Davis, M.A., Wong, E., Pasolli, H.A., Reynolds, A.B., and Fuchs, E. (2006). p120-catenin mediates inflammatory responses in the skin. Cell *124*, 631–644.
5. Elia, L.P., Yamamoto, M., Zang, K., and Reichardt, L.F. (2006). p120 catenin regulates dendritic spine and synapse development through Rho-family GTPases and cadherins. Neuron *51*, 43–56.
6. Myster, S.H., Cavallo, R., Anderson, C.T., Fox, D.T., and Peifer, M. (2003). Drosophila p120catenin plays a supporting role in cell adhesion but is not an essential adherens junction component. J. Cell Biol. *160*, 433–449.
7. Pettitt, J., Cox, E.A., Broadbent, I.D., Flett, A., and Hardin, J. (2003). The Caenorhabditis elegans p120 catenin homologue, JAC-1, modulates cadherin-catenin function during epidermal morphogenesis. J. Cell Biol. *162*, 15–22.
8. Davis, M.A., Ireton, R.C., and Reynolds, A.B. (2003). A core function for p120-catenin in cadherin turnover. J. Cell Biol. *163*, 525–534.
9. Xiao, K., Allison, D.F., Buckley, K.M., Kottke, M., Vincent, P.A., Faundez, V., and Kowalczyk, A.P. (2003). Cellular levels of p120-catenin function as a set point for cadherin expression levels in microvascular endothelial cells. J. Cell Biol. *163*, 535–545.
10. Davis, M.A., and Reynolds, A.B. (2006). Blocked acinar development, E-cadherin reduction, and intraepithelial neoplasia upon ablation of p120-catenin in the mouse salivary gland. Dev. Cell *10*, 21–31.
11. Noren, N.K., Liu, B.P., Burridge, K., and Kreft, B. (2000). p120 catenin regulates the actin cytoskeleton via Rho family GTPases. J. Cell Biol. *150*, 567–580.
12. Grosheva, I., Shtutman, M., Elbaum, M., and Bershadsky, A.D. (2001). p120 catenin affects cell motility via modulation of activity of Rho- family GTPases: a link between cell-cell contact formation and regulation of cell locomotion. J. Cell Sci. *114*, 695–707.
13. Anastasiadis, P.Z., Moon, S.Y., Thoreson, M.A., Mariner, D.J., Crawford, H.C., Zheng, Y., and Reynolds, A.B. (2000). Inhibition of RhoA by p120 catenin. Nat. Cell Biol. *2*, 637–644.
14. Magie, C.R., Pinto-Santini, D., and Parkhurst, S.M. (2002). Rho1 interacts with p120(ctn) and alpha-catenin, and regulates cadherin- based adherens junction components in Drosophila. Development *129*, 3771–3782.
15. Yang, J., Mani, S.A., Donaher, J.L., Ramaswamy, S., Itzykson, R.A., Come, C., Savagner, P., Gitelman, I., Richardson, A., and Weinberg, R.A. (2004). Twist, a master regulator of morphogenesis, plays an essential role in tumor metastasis. Cell *117*, 927–939.
16. Nieman, M.T., Prudoff, R.S., Johnson, K.R., and Wheelock, M.J. (1999). N-cadherin promotes motility in human breast cancer cells regardless of their E-cadherin expression. J. Cell Biol. *147*, 631–644.
17. Tada, T., and Sheng, M. (2006). Molecular mechanisms of dendritic spine morphogenesis. Curr. Opin. Neurobiol. *16*, 95–101.
18. Pacquelet, A., Lin, L., and Rorth, P. (2003). Binding site for p120/delta-catenin is not required for Drosophila E-cadherin function in vivo. J. Cell Biol. *160*, 313–319.
19. Fox, D.T., Homem, C.C., Myster, S.H., Wang, F., Bain, E.E., and Peifer, M. (2005). Rho1 regulates Drosophila adherens junctions independently of p120ctn. Development *132*, 4819–4831.
20. Li, W., Li, Y., and Gao, F.B. (2005). Abelson, enabled, and p120 catenin exert distinct effects on dendritic morphogenesis in Drosophila. Dev. Dyn. *234*, 512–522.

[1]Department of Biology and [2]Lineberger Comprehensive Cancer Center, University of North Carolina Chapel Hill, North Carolina 27599-3280, USA.

DOI: 10.1016/j.cub.2006.11.040

Ecology: A Different Route to Recovery for Coral Reefs

Worldwide, many coral reef ecosystems have undergone regime shifts, changing from domination by coral to domination by algae. New work indicates that the return path is surprisingly different from the forward one.

Lance Gunderson

Throughout the tropics, direct and indirect human activities have led to dramatic changes in coral reef ecosystems. Sudden shifts from coral-dominated systems to either algae-dominated systems or barren landscapes have been observed around the world, and described as the coral reef crisis [1]. Much research has gone into understanding the interaction between human drivers and the ecology of reef deterioration [1–3]. A new paper by Bellwood *et al.* [4], published recently in *Current Biology*, indicates that not only are some of these changes reversible, but that the return path occurs through the functional role played by an unexpected species.

Coral reefs are one of the most productive ecosystems on the planet, providing a wide array of goods and services for humans [1]. In providing these resources, coral reefs are affected by a wide range of human activities, including the harvesting of invertebrates and vertebrates for consumption and for economic markets, and the mining of mineral resources. Land use changes also modify the water quality of reefs through increasing silt and nutrient concentrations [2,3]. Anticipated climatic changes, such as increased temperatures with global warming, are expected to profoundly change reef structure and function. These and other unforeseen changes threaten the characteristics of the reef ecosystem that humans have exploited for millennia; some have characterized these changes as the coral reef crisis [1].

The observed changes in reefs are related to their resilience. In ecology, the term resilience is used to describe how a system responds to external perturbations. Some consider resilience to be the rate at which a system recovers following a perturbation. Holling [5], however, argued that disturbances cause many ecosystems to change into a fundamentally different system, with different structures, feedbacks and controls. In this case, resilience is the property that mediates the transitions among